Jain, S.C., Shinde, U., Li, Y., Inouye, M., and Berman, H.M. (1998). J. Mol. Biol. *284*, 137–144.

Lin, L., Sohar, I., Lackland, H., and Lobel, P. (2001). J. Biol. Chem. *276*, 2249–2255.

Oda, K., Sugitani, M., Fukuhara, K., and Murao, S. (1987). Biochim. Biophys. Acta *923*, 463–469.

Rawlings, N.D., and Barrett, A.J. (1999). Biochim. Biophys. Acta *1429*, 496–500.

Sleat, D.E., Donnelly, R.J., Lackland, H., Liu, C.G., Sohar, I., Pullarkat, R.K., and Lobel, P. (1997). Science *277*, 1802–1805.

Wlodawer, A., Li, M., Dauter, Z., Gustchina, A., Uchida, K., Oyama, H., Dunn, B.M., and Oda, K. (2001). Nat. Struct. Biol. *8*, 442–446.

Wlodawer, A., Li, M., Gustchina, A., Oyama, H., Dunn, B.M., and Oda, K. (2003). Acta Biochim. Polon. *50*, 81–102.

Wlodawer, A., Li, M., Gustchina, A., Oyama, H., Oda, K., Beyer, B.B., Clemente, J., and Dunn, B.M. (2004). Biochem. Biophys. Res. Commun. *314*, 638–645.

Structure, Vol. 12, July, 2004, ©2004 Elsevier Ltd. All rights reserved. DOI 10.1016/j.str.2004.06.009

Visualizing the House from the Brick

In this issue of *Structure*, Dokland et al. (2004) present the crystal structure of West Nile Virus capsid protein and open a new perspective on the possible structure of the flavivirus capsid.

The structures of virus capsid proteins were once fairly predictable. The occurrence of a common fold in the first virus structures solved by X-ray crystallography led to the inference that it might be a consistent feature of virus structures. Membrane viruses, as is so often the case, proved exceptions to this generalization. The prediction that the alphavirus capsid protein would be similar to the nonenveloped ones (Fuller and Argos, 1987) was proven false by the Sindbis capsid protein structure (Choi et al., 1991). Indeed, a serine protease proved a better model for the alphavirus capsid.

Alphavirus and flaviviruses are two icosahedral membrane viruses bearing type two fusion proteins with the same fold (Lescar et al., 2001; Rey et al., 1995). The capsid proteins of the alphaviruses form their icosahedral shells prior to the interaction with the envelope proteins. This interaction and lateral interactions between spikes drives virus budding from the plasma membrane. The nucleocapsid can be isolated from infected cells and from virions. Image reconstructions from cryo-electron micrographs established the organization of the complementary T = 4 nucleocapsid and envelope protein layers (Mancini et al., 2000). The capsid protein contains a positively charged tail that interacts with the viral RNA and is toward the center of the nucleocapsid (Choi et al., 1991).

The similarity between the fusion proteins does not extend to the capsid of the flaviviruses. Icosahedral capsids are difficult to isolate from virions or infected cells. Image reconstruction from cryo-electron micrographs of mature or immature viruses show well-ordered envelope proteins (Kuhn et al., 2002; Zhang et al., 2003) but reveal no ordered density for the capsid. Kuhn et al. (2002) suggested that the flavivirus capsid does not share the organization of the surface proteins. The absence of a structure for the flavivirus capsid makes the interpretation of the capsid protein structure akin to inferring the organization of a house from a brick. Nevertheless, such hypotheses are the only route toward understanding these important structures.

Ma et al. (2004) used an elegant NMR method to determine the structure of a dimer of the Dengue virus capsid protein. They demonstrated that the flavivirus capsid protein is a novel fold. The monomer comprises a core of three helices, α1–α3, with a fourth, α4, extending from the core. The dimer showed an uneven distribution of charge. They proposed that the dimer would be oriented in the virus with its positively charged regions extending centrally to interact with the RNA and the hydrophobic region interacting with the membrane (Figure 1).

Dokland et al. (2004) determined the crystal structure of the capsid protein of the Kunjin strain of West Nile virus. Their structure confirms the dimer structure presented by Ma et al. (2004) and revealed flexibility in the α1 helix. The structure also revealed that the dimers form tetramers in the crystal. This shields the hydrophobic regions during crystal formation and provides a highly positively charged surface that could interact with the viral genome (Figure 1). They point out that the tetramers form filaments reminiscent of those formed by the HEAT motifs in many nucleic acid interacting proteins. The ribbons formed in the crystal illustrate the types of interactions that could be used to build the more complex network required to construct a capsid. An order-disorder transition of the α1 helix appears to modulate these interactions as is common in virus capsid assembly.

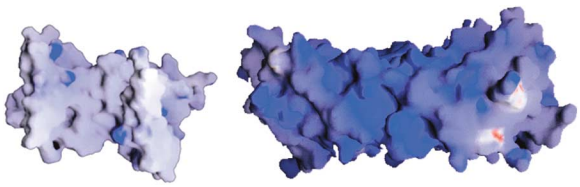

Figure 1. Surface Potential of Flavivirus Capsid Oligomers

The GRASP (Nicholls et al., 1991) representation of the surface potential for the dengue virus capsid protein dimer (left) and the West Nile virus capsid protein tetramer (right). The face proposed to be against the membrane is facing the viewer in both images.

If their surmise is correct, Dokland et al. (2004) will have used their structure of a tetramer to bring us closer to an image of the assembled flavivirus capsid.

Stephen D. Fuller
Division of Structural Biology
Wellcome Trust Centre for Human Genetics
University of Oxford
Roosevelt Drive
Headington, Oxford OX3 7BN
United Kingdom

Selected Reading

Choi, H.K., Tong, L., Minor, W., Dumas, P., Boege, U., Rossmann, M.G., and Wengler, G. (1991). Nature *354*, 37–43.

Dokland, T., Walsh, M., Mackenzie, J.M., Kromykh, A.A., Ee, K.-H., and Wang, S. (2004). Structure *12*, this issue, 1157–1163.

Fuller, S.D., and Argos, P. (1987). Eur. Mol. Biol. Org. J. *6*, 1099–1105.

Kuhn, R.J., Zhang, W., Rossmann, M.G., Pletnev, S.V., Corver, J., Lenches, E., Jones, C.T., Mukhopadhyay, S., Chipman, P.R., Strauss, E.G., et al. (2002). Cell *108*, 717–725.

Lescar, J., Roussel, A., Wien, M.W., Navaza, J., Fuller, S.D., Wengler, G., and Rey, F.A. (2001). Cell *105*, 137–148.

Ma, L., Jones, C.T., Groesch, T.D., Kuhn, R.J., and Post, C.B. (2004). Proc. Natl. Acad. Sci. USA *101*, 3414–3419.

Mancini, E.J., Clarke, M., Gowen, B.E., Rutten, T., and Fuller, S.D. (2000). Mol. Cell *5*, 255–266.

Nicholls, A., Sharp, K.A., and Honig, B. (1991). *11*, 281–296.

Rey, F.A., Heinz, F.X., Mandl, C., Kunz, C., and Harrison, S.C. (1995). Nature *375*, 291–298.

Zhang, Y., Corver, J., Chipman, P.R., Zhang, W., Pletnev, S.V., Sedlak, D., Baker, T.S., Strauss, J.H., Kuhn, R.J., and Rossmann, M.G. (2003). EMBO J. *22*, 2604–2613.

Structure, Vol. 12, July, 2004, DOI 10.1016/j.str.2004.06.008

siRNAs at RISC

A recent report (Ma et al., 2004) describes the crystal structure of a "mini-siRNA" bound to a PAZ domain.

RNA interference (RNAi) has taken experimental biology by storm in recent years, as RNAi has quickly evolved from a biological curiosity in plants and nematodes into a powerful gene knock down technology in many eukaryotic systems, including worms, flies, and mammals. This evolutionary conserved pathway (reviewed in Hannon [2002]) is triggered in response to exogenous dsRNA introduced to cells, for example, by viral infection or transfection of in vitro synthesized dsRNA, as well as by the expression of endogenously encoded RNAi triggers, microRNAs (miRNA). Depending on the nature of the dsRNA trigger and the biological system in which it is expressed, RNAi results in the downregulation of a homologous target gene through the cleavage, translational repression, or transcriptional inhibition of its mRNA. The RNAi machinery has been linked to the establishment of heterochromatin and proper centromere function in fission yeast (Hall et al., 2002; Volpe et al., 2002) and appears to have prominent roles during the development of multicellular eukaryotic organisms through action of miRNAs (Carrington and Ambros, 2003).

Two core proteins are universally associated with RNAi-related silencing phenomena: Dicer and Argonaute. Dicer is an RNase III type nuclease that cleaves dsRNA to generate short interfering RNAs (siRNAs), which are dsRNA of 19–24 nucleotides with two-nucleotide 3′ overhangs and a 5′ phosphate at each end (Bernstein et al., 2001). Dicer also cleaves short hairpin miRNA precursors, which are in turn produced by a related nuclease called Drosha, to generate miRNAs (Lee et al., 2003). These structures, siRNAs or miRNAs, are loaded into an effector complex called RISC, the RNA-induced silencing complex, to select its target, a homologous mRNA for destruction in the case of siRNAs or, for miRNAs, blocking mRNA translation (Carrington and Ambros, 2003; Hammond et al., 2001; Martinez et al., 2002). RISC contains an Argonaute protein as its signature component, which has two characteristic domains, PAZ, also found in Dicer family proteins, and PIWI (Carmell et al., 2002; Cerutti et al., 2000). In *Drosophila*, loading appears to occur via an intermediate complex, the RISC loading complex that contains Dicer-2 and R2D2 (Liu et al., 2003).

Though there has been remarkable progress in defining the cast of characters involved in the RNAi pathway, we still do not understand how they work at the molecular level to orchestrate this process. This is where the structural biologists are starting to make an impact. Our first glimpse into the molecular machinery of RNAi came with three separate reports of the three-dimensional structures of the PAZ domains of Argonaute proteins (Lingel et al., 2003; Song et al., 2003; Yan et al., 2003). These implicated the PAZ domain as an RNA binding module for Argonautes. It was also suggested that the PAZ domain acts as a specificity determinant for siRNA or pre-miRNA structures since it appears to bind to the characteristic two-nucleotide 3′ overhang of siRNAs (Song et al., 2003). Now, Patel and his colleagues have determined the crystal structure of the human Argonaute1 (eIF2c1) PAZ with a 9-mer RNA that formed a "mini siRNA," an siRNA look-alike with a 7-base pair A-form duplex and a two-nucleotide 3′ overhang at each end (Ma et al., 2004). As for the two fly proteins, the human Ago1 PAZ domain adopts an OB-like β-barrel fold with an $\alpha\beta$ module attachment and a cleft in between. The cleft was implicated by mutagenesis and NMR chemical shift perturbations in RNA binding (Lingel et al., 2003; Song et al., 2003; Yan et al., 2003). Now we can see how the siRNA actually binds. The RNA strand